

SUBSTITUTE SEQUENCE LISTING

<110> Degussa AG

<120> Cyanide-tolerant nitrile hydratases

<130> 040061

<140> US/10/598,873

<141> 2006-09-14

<160> 14

<170> PatentIn version 3.3

<210> 1

<211> 6828

<212> DNA

<213> Pseudomonas marginalis

<220>

<221> CDS

<222> (25)..(609)

<223> Coding region of alpha-subunit gene

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<221> CDS

<222> (650)..(1312)

<223> Coding region of beta-subunit gene

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<221> gene

<222> (1309)..(2577)

<223> Gene of activator protein

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51

gaa aga gcc tgg gca ttg ttt caa gtc ctc aag agc aag gaa ctc atc  
Glu Arg Ala Trp Ala Leu Phe Gln Val Leu Lys Ser Lys Glu Leu Ile  
10 15 20 25

99

ccg gag ggc tat gtc gag cag ctc acg caa ttg atg gag cac ggc tgg  
Pro Glu Gly Tyr Val Glu Gln Leu Thr Gln Leu Met Glu His Gly Trp  
30 35 40

147

agc ccc gag aac ggc gcc cgt gtg gtg gcc aag gcg tgg gtc gat ccg  
Ser Pro Glu Asn Gly Ala Arg Val Val Ala Lys Ala Trp Val Asp Pro  
45 50 55

195

cag ttc cgg gca ctg ttg ctc aag gac ggc acc gcg gcc tgc gcc cag  
Gln Phe Arg Ala Leu Leu Lys Asp Gly Thr Ala Ala Cys Ala Gln  
60 65 70

243

ttc ggc tac acc ggc ccc cag ggc gaa tac atc gtt gcc ctg gag gat

291

Phe	Gly	Tyr	Thr	Gly	Pro	Gln	Gly	Glu	Tyr	Ile	Val	Ala	Leu	Glu	Asp			
75					80						85							
acg	ccg	acg	ctg	aag	aac	gtg	att	gtc	tgc	agc	ctg	tgc	tcc	tgc	acc		339	
Thr	Pro	Thr	Leu	Lys	Asn	Val	Ile	Val	Cys	Ser	Leu	Cys	Ser	Cys	Thr			
90						95				100					105			
aac	tgg	ccg	gtc	ctc	ggc	ctg	cca	ccg	gag	tgg	tac	aag	ggt	ttc	gag		387	
Asn	Trp	Pro	Val	Leu	Gly	Leu	Pro	Pro	Glu	Trp	Tyr	Lys	Gly	Phe	Glu			
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ttc	cgc	gca	cgc	ctg	gtc	cg	gag	ggg	cgc	acg	gta	ctg	cgc	gag	ctg		435	
Phe	Arg	Ala	Arg	Leu	Val	Arg	Glu	Gly	Arg	Thr	Val	Leu	Arg	Glu	Leu			
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ggg	acg	gag	ttg	ccc	cg	gac	atg	gtg	gtc	aag	gtc	tgg	gac	acc	agc		483	
Gly	Thr	Glu	Leu	Pro	Arg	Asp	Met	Val	Val	Lys	Val	Trp	Asp	Thr	Ser			
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gcc	gaa	agc	cgc	tac	ctg	gtg	ctg	cg	gtc	agg	ccg	gaa	ggc	tca	gaa		531	
Ala	Glu	Ser	Arg	Tyr	Leu	Val	Leu	Pro	Val	Arg	Pro	Glu	Gly	Ser	Glu			
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cac	atg	agc	gaa	gag	cag	ctt	caa	g	cg	ctg	gtg	acc	aaa	gac	gtg	ctg		579
His	Met	Ser	Glu	Glu	Gln	Leu	Gln	Ala	Leu	Val	Thr	Lys	Asp	Val	Leu			
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atc	ggc	gtc	gcc	ctg	ccc	cg	gtg	gg	tc	gaacaacacc	tcatcatcg						629	
Ile	Gly	Val	Ala	Leu	Pro	Arg	Val	Gly										
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Met	Asp	Gly	Phe	His	Asp	Leu	Gly	Gly	Phe	Gln								
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ggc	ttt	gga	aaa	gtc	cct	cac	acc	atc	aac	agc	ctg	agc	tac	aaa	cag		730	
Gly	Phe	Gly	Lys	Val	Pro	His	Thr	Ile	Asn	Ser	Leu	Ser	Tyr	Lys	Gln			
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Val	Phe	Lys	Gln	Asp	Trp	Glu	His	Leu	Ala	Tyr	Ser	Leu	Met	Phe	Ile			
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Gly	Ala	Asp	His	Leu	Lys	Lys	Phe	Ser	Val	Asp	Glu	Val	Arg	His	Ala			
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gtc	gaa	cg	ctg	gat	gtg	cg	cag	cat	gtc	ggc	acc	cag	tac	tac	gaa		874	
Val	Glu	Arg	Leu	Asp	Val	Arg	Gln	His	Val	Gly	Thr	Gln	Tyr	Tyr	Glu			
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cgc	tac	gtc	atc	cg	acc	ggc	acc	ctg	gtc	gaa	acc	ggc	gtg	atc			922	
Arg	Tyr	Val	Ile	Ala	Thr	Ala	Thr	Leu	Leu	Val	Glu	Thr	Gly	Val	Ile			
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Thr	Gln	Ala	Glu	Leu	Asp	Gln	Ala	Leu	Gly	Ser	His	Phe	Lys	Leu	Ala			
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Asn Pro Ala His Ala Glu Gly Arg Pro Ala Ile Thr Gly Arg Pro Pro  
305 310 315

ttc gag gtg ggg gat cgg gtg gtg gtg cga gac gaa tat gtg gct gga Phe Glu Val Gly Asp Arg Val Val Val Arg Asp Glu Tyr Val Ala Gly 320 325 330	1066
cac atc cgc atg ccc gcc tac gtg cgc ggc aag gaa ggc gtg gtc ctg His Ile Arg Met Pro Ala Tyr Val Arg Gly Lys Glu Gly Val Val Leu 335 340 345	1114
cac cgc acg tca gag aaa tgg ccg ttc ccc gac gca atc ggg cat ggc His Arg Thr Ser Glu Lys Trp Pro Phe Pro Asp Ala Ile Gly His Gly 350 355 360 365	1162
gat gta agc gca gcc cat caa ccc acc tac cac gtc gag ttc gcc gtg Asp Val Ser Ala Ala His Gln Pro Thr Tyr His Val Glu Phe Ala Val 370 375 380	1210
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Leu Thr Gln Leu Met Glu His Gly Trp Ser Pro Glu Asn Gly Ala Arg  
35 40 45

Val Val Ala Lys Ala Trp Val Asp Pro Gln Phe Arg Ala Leu Leu Leu  
50 55 60

Lys Asp Gly Thr Ala Ala Cys Ala Gln Phe Gly Tyr Thr Gly Pro Gln  
65 70 75 80

Gly Glu Tyr Ile Val Ala Leu Glu Asp Thr Pro Thr Leu Lys Asn Val  
85 90 95

Ile Val Cys Ser Leu Cys Ser Cys Thr Asn Trp Pro Val Leu Gly Leu  
100 105 110

Pro Pro Glu Trp Tyr Lys Gly Phe Glu Phe Arg Ala Arg Leu Val Arg  
115 120 125

Glu Gly Arg Thr Val Leu Arg Glu Leu Gly Thr Glu Leu Pro Arg Asp  
130 135 140

Met Val Val Lys Val Trp Asp Thr Ser Ala Glu Ser Arg Tyr Leu Val  
145 150 155 160

Leu Pro Val Arg Pro Glu Gly Ser Glu His Met Ser Glu Glu Gln Leu  
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Val Gly

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<213> *Pseudomonas marginalis*

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Trp Glu His Leu Ala Tyr Ser Leu Met Phe Ile Gly Ala Asp His Leu  
35 40 45

Lys Lys Phe Ser Val Asp Glu Val Arg His Ala Val Glu Arg Leu Asp  
50 55 60

Val Arg Gln His Val Gly Thr Gln Tyr Tyr Glu Arg Tyr Val Ile Ala  
65 70 75 80

Thr Ala Thr Leu Leu Val Glu Thr Gly Val Ile Thr Gln Ala Glu Leu

85

90

95

Asp Gln Ala Leu Gly Ser His Phe Lys Leu Ala Asn Pro Ala His Ala  
 100 105 110

Glu Gly Arg Pro Ala Ile Thr Gly Arg Pro Pro Phe Glu Val Gly Asp  
 115 120 125

Arg Val Val Val Arg Asp Glu Tyr Val Ala Gly His Ile Arg Met Pro  
 130 135 140

Ala Tyr Val Arg Gly Lys Glu Gly Val Val Leu His Arg Thr Ser Glu  
 145 150 155 160

Lys Trp Pro Phe Pro Asp Ala Ile Gly His Gly Asp Val Ser Ala Ala  
 165 170 175

His Gln Pro Thr Tyr His Val Glu Phe Ala Val Lys Asp Leu Trp Gly  
 180 185 190

Asp Ala Ala Asp Glu Gly Phe Val Val Val Asp Leu Phe Glu Ser Tyr  
 195 200 205

Leu Asp Lys Ala Ala Gly Ala Arg Ala Val Asn Pro  
 210 215 220

<210> 4  
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 <212> DNA  
 <213> Pseudomonas marginalis

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 <222> (1)..(1269)  
 <223> Coding region of activator protein gene

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ggc ttc ctc ggc gcc ggc aag acc acc ctg ctc aac cac atc ctg cgc 96  
 Gly Phe Leu Gly Ala Gly Lys Thr Thr Leu Leu Asn His Ile Leu Arg  
 20 25 30

aat cgc gaa ggc ctg cgc gtg gcc gtc atc gtc aat gac atg agc gaa 144  
 Asn Arg Glu Gly Leu Arg Val Ala Val Ile Val Asn Asp Met Ser Glu  
 35 40 45

gtc aat atc gat gcc gaa gag gtg cag cgc gat gtc gcg ctg cac cgt 192  
 Val Asn Ile Asp Ala Glu Glu Val Gln Arg Asp Val Ala Leu His Arg

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65 70 75 80			
ctg cgc gcc gat ttg ctc gag cag atc agc atg ctc gca cgc caa cag Leu Arg Ala Asp Leu Leu Glu Gln Ile Ser Met Leu Ala Arg Gln Gln	288		
85 90 95			
cgt ttc gat tac ctg ctg att gaa tcc acg ggg atc tcc gag ccg atg Arg Phe Asp Tyr Leu Leu Ile Glu Ser Thr Gly Ile Ser Glu Pro Met	336		
100 105 110			
ccg gtc gcg gag acg ttc gcc ttc ctt gac gct gat ggc ttc agc ctc Pro Val Ala Glu Thr Phe Ala Phe Leu Asp Ala Asp Gly Phe Ser Leu	384		
115 120 125			
agc gaa ctg gcg cgc ctg gac acc ttg gtg acg gtg gtc gat ggc agt Ser Glu Leu Ala Arg Leu Asp Thr Leu Val Thr Val Val Asp Gly Ser	432		
130 135 140			
cgt ttc cag gaa ctg ctc gaa tcg ccg cac acc gtt gac cag gat gac Arg Phe Gln Glu Leu Leu Glu Ser Pro His Thr Val Asp Gln Asp Asp	480		
145 150 155 160			
gcc acg cca gac gca ccc aag cgc cac ctg gcc gat ctg ctg atc gaa Ala Thr Pro Asp Ala Pro Lys Arg His Leu Ala Asp Leu Leu Ile Glu	528		
165 170 175			
cag gtg gag tac gcc aac gtc att ctc gtc aat aag ctg gat ctg atc Gln Val Glu Tyr Ala Asn Val Ile Leu Val Asn Lys Leu Asp Leu Ile	576		
180 185 190			
gat gca gcg cag tat cag gcc gtg cag gcg atc ctc aca ggc ctt aac Asp Ala Ala Gln Tyr Gln Ala Val Gln Ala Ile Leu Thr Gly Leu Asn	624		
195 200 205			
ccg acg gcg cgg atc atg ccg atg gcc cac ggt aac atc cca tca gcc Pro Thr Ala Arg Ile Met Pro Met Ala His Gly Asn Ile Pro Ser Ala	672		
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agc ctg ctc ggc acc cat ctg ttt gat tta ccc agc ctc gcg gcg tcg Ser Leu Leu Gly Thr His Leu Phe Asp Leu Pro Ser Leu Ala Ala Ser	720		
225 230 235 240			
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275 280 285			
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290 295 300			

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Leu Glu Thr Gly Leu Leu Val Gln Ser Gly Lys Arg Phe Gln Trp Asp	
305 310 315 320	
tat gtc ggg cgcc tgg tgg aac ttc atc gag ccg tcg caa tgg ccc cgg	1008
Tyr Val Gly Arg Trp Trp Asn Phe Ile Glu Pro Ser Gln Trp Pro Arg	
325 330 335	
gac gaa tac cgg ctg cag ggc atc agg gcc aaa tgg gac agc gtg gtc	1056
Asp Glu Tyr Arg Leu Gln Gly Ile Arg Ala Lys Trp Asp Ser Val Val	
340 345 350	

ggc gac tgc cgg cag gag ttg gtg ttt atc ggc cag ggc ctc gac acc	1104		
Gly Asp Cys Arg Gln Glu Leu Val Phe Ile Gly Gln Gly Leu Asp Thr			
355	360	365	
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Asp Ala Leu Gln Arg Glu Leu Asp His Cys Leu Leu Ser Ala Gln Glu			
370	375	380	
atc gcc gcc ggc cca ctg gcc tgg caa gcg ctg cca ggg gcg acc gcc	1200		
Ile Ala Ala Gly Pro Leu Ala Trp Gln Ala Leu Pro Gly Ala Thr Ala			
385	390	395	400
ttt gac cga cag acc ctt gcc cgc ccc cca cac agc cca tgg cga ttg	1248		
Phe Asp Arg Gln Thr Leu Ala Arg Pro Pro His Ser Pro Trp Arg Leu			
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Pro Pro Phe Asp Pro Arg			
420			

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Asn Arg Glu Gly Leu Arg Val Ala Val Ile Val Asn Asp Met Ser Glu  
35 40 45

Val Asn Ile Asp Ala Glu Glu Val Gln Arg Asp Val Ala Leu His Arg  
50 55 60

Gly Arg Asp Glu Leu Ile Glu Met Ser Asn Gly Cys Ile Cys Cys Thr  
65 70 75 80

Leu Arg Ala Asp Leu Leu Glu Gln Ile Ser Met Leu Ala Arg Gln Gln  
85 90 95

Arg Phe Asp Tyr Leu Leu Ile Glu Ser Thr Gly Ile Ser Glu Pro Met  
100 105 110

Pro Val Ala Glu Thr Phe Ala Phe Leu Asp Ala Asp Gly Phe Ser Leu  
115 120 125

Ser Glu Leu Ala Arg Leu Asp Thr Leu Val Thr Val Val Asp Gly Ser  
130 135 140

Arg Phe Gln Glu Leu Leu Glu Ser Pro His Thr Val Asp Gln Asp Asp  
145 150 155 160

Ala Thr Pro Asp Ala Pro Lys Arg His Leu Ala Asp Leu Leu Ile Glu  
165 170 175

Gln Val Glu Tyr Ala Asn Val Ile Leu Val Asn Lys Leu Asp Leu Ile  
180 185 190

Asp Ala Ala Gln Tyr Gln Ala Val Gln Ala Ile Leu Thr Gly Leu Asn  
195 200 205

Pro Thr Ala Arg Ile Met Pro Met Ala His Gly Asn Ile Pro Ser Ala  
210 215 220

Ser Leu Leu Gly Thr His Leu Phe Asp Leu Pro Ser Leu Ala Ala Ser  
225 230 235 240

Pro Gly Trp Met Arg Lys Met Glu Ala Ala Asp Ala Pro Ala Ser Glu  
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Ser Asp Thr Tyr Gly Val Thr Ser Trp Val Tyr Arg Glu Arg Ala Pro  
260 265 270

Phe His Pro Gln Arg Leu Leu Asp Phe Leu Gln Gln Pro Trp Cys Asn  
275 280 285

Gly Arg Leu Leu Arg Ser Lys Gly Tyr Phe Trp Leu Ala Ser Arg His  
290 295 300

Leu Glu Thr Gly Leu Leu Val Gln Ser Gly Lys Arg Phe Gln Trp Asp  
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Tyr Val Gly Arg Trp Trp Asn Phe Ile Glu Pro Ser Gln Trp Pro Arg  
325 330 335

Asp Glu Tyr Arg Leu Gln Gly Ile Arg Ala Lys Trp Asp Ser Val Val  
340 345 350

Gly Asp Cys Arg Gln Glu Leu Val Phe Ile Gly Gln Gly Leu Asp Thr  
355 360 365

Asp Ala Leu Gln Arg Glu Leu Asp His Cys Leu Leu Ser Ala Gln Glu  
370 375 380  
Ile Ala Ala Gly Pro Leu Ala Trp Gln Ala Leu Pro Gly Ala Thr Ala  
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Pro Pro Phe Asp Pro Arg  
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<210> 6  
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<223> Coding region of alpha-subunit gene

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<221> CDS  
<222> (624)..(1286)  
<223> Coding region of beta-subunit gene

<220>  
<221> gene  
<222> (1283)..(2371)  
<223> Gene of activator protein

<400> 6  
atg acg gca act tca acc cct ggt gag cgg gca cgc gca ttg ttt gca 48  
Met Thr Ala Thr Ser Thr Pro Gly Glu Arg Ala Arg Ala Leu Phe Ala  
1 5 10 15

gtg ctc aag cgc aaa gac ctc atc ccc gag ggc tac atc gaa cag ctc 96  
Val Leu Lys Arg Lys Asp Leu Ile Pro Glu Gly Tyr Ile Glu Gln Leu  
20 25 30

acc cag ctg atg gaa cac ggc tgg agc ccg gaa aac ggc gcg cgc atc 144  
Thr Gln Leu Met Glu His Gly Trp Ser Pro Glu Asn Gly Ala Arg Ile  
35 40 45

gtc gcc aag gcc tgg gtc gat ccg cag ttt cgc gag ctg ctg ctc aag 192  
Val Ala Lys Ala Trp Val Asp Pro Gln Phe Arg Glu Leu Leu Leu Lys  
50 55 60

gac ggt acg gcc gcc tgc gcc cag ttc ggc ttc acc ggc cca caa ggc 240  
Asp Gly Thr Ala Ala Cys Ala Gln Phe Gly Phe Thr Gly Pro Gln Gly  
65 70 75 80

gaa tac atc gtc gcc ctg gaa gac acc ccg cag ttg aaa aac gtg atc 288  
Glu Tyr Ile Val Ala Leu Glu Asp Thr Pro Gln Leu Lys Asn Val Ile  
85 90 95

gtc tgt agc ctg tgc tcc tgc acg aac tgg ccg gtg ctg ggc ctg cca 336

Val Cys Ser Leu Cys Ser Cys Thr Asn Trp Pro Val Leu Gly Leu Pro			
100	105	110	
cct gag tgg tac aag ggc ttc gag ttc cgt gcg cggttg gtc cgg gag			384
Pro Glu Trp Tyr Lys Gly Phe Glu Phe Arg Ala Arg Leu Val Arg Glu			
115	120	125	
ggg cgc acg gta ttg cgc gag ctg ggc acc gag ttg ccc ggc gac atg			432
Gly Arg Thr Val Leu Arg Glu Leu Gly Thr Glu Leu Pro Gly Asp Met			
130	135	140	
gtg gtc aag gtc tgg gac acc agc gct gaa agc cgc tac ctg gtg ctg			480
Val Val Lys Val Trp Asp Thr Ser Ala Glu Ser Arg Tyr Leu Val Leu			
145	150	155	160
ccg caa cga cca gcg ggc tca gag cat atg agc gaa gag cag ttg cgg			528
Pro Gln Arg Pro Ala Gly Ser Glu His Met Ser Glu Glu Gln Leu Arg			
165	170	175	
caa ctg gtc acc aag gac gtg ctg atc ggc gtc gcc ctg ccc cgc gtt			576
Gln Leu Val Thr Lys Asp Val Leu Ile Gly Val Ala Leu Pro Arg Val			
180	185	190	
ggc tga gcaaggccgc ccaacccat tcaacttccg gagtgttcaa t atg gat ggc			632
Gly		Met Asp Gly	
	195		
ttt cac gat ctc ggc ggt ttc cag ggc ttt ggc aaa gtg ccc cac cgc			680
Phe His Asp Leu Gly Gly Phe Gln Gly Phe Gly Lys Val Pro His Arg			
200	205	210	
atc aac agc ctg agc tac aag cag gtg ttc aag cag gac tgg gaa cac			728
Ile Asn Ser Leu Ser Tyr Lys Gln Val Phe Lys Gln Asp Trp Glu His			
215	220	225	
ctg gcc tac agc ctg atg ttc atc ggc gtc gac cac ctg aac aag ttc			776
Leu Ala Tyr Ser Leu Met Phe Ile Gly Val Asp His Leu Asn Lys Phe			
230	235	240	
agc gtc gac gaa ata cgt cat gcc gtc gaa cgc att gac gtg cgc cag			824
Ser Val Asp Glu Ile Arg His Ala Val Glu Arg Ile Asp Val Arg Gln			
245	250	255	260
cac gtc ggc acc gaa tac tac gaa cgt tat gtg atc gcc act gcc acg			872
His Val Gly Thr Glu Tyr Tyr Glu Arg Tyr Val Ile Ala Thr Ala Thr			
265	270	275	
ctg ctg gtc gaa aca ggc gtc atc acc cag gcc gaa ctg gat gaa gca			920
Leu Leu Val Glu Thr Gly Val Ile Thr Gln Ala Glu Leu Asp Glu Ala			
280	285	290	
ctc ggc tcg cac ttc aag ctg gcc aac ccc gcc cat gcg caa ggg cgt			968
Leu Gly Ser His Phe Lys Leu Ala Asn Pro Ala His Ala Gln Gly Arg			
295	300	305	
gct gca att atc ggg cga gcg cct ttt gaa gtg ggc gat cgg gtc atc			1016
Ala Ala Ile Ile Gly Arg Ala Pro Phe Glu Val Gly Asp Arg Val Ile			
310	315	320	
gta cgc gat gaa tac gtg gcc ggg cat gtg cgc atg cct gca tac gtg			1064
Val Arg Asp Glu Tyr Val Ala Gly His Val Arg Met Pro Ala Tyr Val			

325	330	335	340	
cgc ggc aag caa ggc gta gtg ctg cac cg <sup>g</sup> acc act gaa cag tgg cc <sup>g</sup>				1112
Arg Gly Lys Gln Gly Val Val Leu His Arg Thr Thr Glu Gln Trp Pro				
345	350	355		
ttt ccg gac g <sup>c</sup> att ggc cat ggc gac cag agc gct g <sup>c</sup> cat caa cc <sup>g</sup>				1160
Phe Pro Asp Ala Ile Gly His Gly Asp Gln Ser Ala Ala His Gln Pro				
360	365	370		
acc tac cat gtc gag ttc cgc gtg cg <sup>g</sup> gac ctg tgg ggc gat gcc gca				1208
Thr Tyr His Val Glu Phe Arg Val Arg Asp Leu Trp Gly Asp Ala Ala				
375	380	385		
gac gac ggc ctg gtg gtg gta gac ctg ttc gaa agc tat ctg gac agg				1256
Asp Asp Gly Leu Val Val Asp Leu Phe Glu Ser Tyr Leu Asp Arg				
390	395	400		
gtc gaa agc ccg cga gtg gtg cgc gca tga gtgccggcgc ccaggcaggc				1306
Val Glu Ser Pro Arg Val Val Arg Ala				
405	410			
cggtgtccgg tgacggcttc tt <sup>c</sup> aggcttc ctcggcgcag gcaagaccac cctgctcaac				1366
cacatcctgc gcaaccgcca gggcctgaag gtggcggtta tcgtcaatga catgagc <sup>g</sup> ag				1426
gtcaacatcg atgccc <sup>c</sup> cca ggtccagcgc gacgttgcgc tgtatcg <sup>t</sup> gg ccaggatgaa				1486
ttgatagaga tgagcaacgg ctgtatctgc tgcaccctgc gcgcgcacct gcttgagcag				1546
atcagcgcgc tggcgcgcca gcagcg <sup>t</sup> tc gattacctgt tgatcgagtc caccggatt				1606
tccgagccga tgccagtcgc cgagac <sup>c</sup> ttt gccttctcg acgccaacgg tttcagc <sup>c</sup> tc				1666
agcgaactgg cgccggctgga tacgctgg <sup>t</sup> g acgg <sup>t</sup> ggcgtcg atgccagcca gttcatggcc				1726
atgctcgact ctccc <sup>a</sup> aac cgtcg <sup>c</sup> gcgg gccgacgtca ccacggatga cagcaggcgc				1786
ccgctggccg atctgctgat cgagcagg <sup>t</sup> tc gagtatgcca atgtgattct ggtcaacaaa				1846
cgcgac <sup>c</sup> tgg tcgacgaggc gcagtaccag gc <sup>c</sup> c <sup>c</sup> tcgagg cagttctcg <sup>c</sup> cggc <sup>c</sup> taat				1906
ccaggcgcac agatcctgcc gatgg <sup>t</sup> ggcc ggcaacgtcg ccctgtcgag cgtccttggt				1966
acccagctgt tcgatttgcc cagc <sup>c</sup> ttgcc gcagcgcccc gctggatgaa acagatggac				2026
g <sup>c</sup> gcacgaca ccccggccgg cgagtcgcag acctatggcg tgacgtcatg ggtgtaccga				2086
gcgcgcgc <sup>c</sup> cc cgttccatcc gcaacgcttg cttgattttc tcgcccggcc ctggcgcgac				2146
ggccgtcttc tg <sup>c</sup> gcagcaa aggttatttc tggcttgcca gccgccac <sup>c</sup> cg gaaatcg <sup>c</sup> gc				2206
ttgctgg <sup>t</sup> ac acagcggcca gcagttcaa tggactatg ttggccattg gtggacttc				2266
atcgacacgt cacagtggcc acaggacaag tatcgctgc agggcatcat ggccaagtgg				2326
gacagcatcg tcggcgactg ccgacaggag ctgaaaagct tatga				2371

<210> 7  
<211> 193  
<212> PRT  
<213> Pseudomonas putida

<400> 7

Met Thr Ala Thr Ser Thr Pro Gly Glu Arg Ala Arg Ala Leu Phe Ala  
1 5 10 15

Val Leu Lys Arg Lys Asp Leu Ile Pro Glu Gly Tyr Ile Glu Gln Leu  
20 25 30

Thr Gln Leu Met Glu His Gly Trp Ser Pro Glu Asn Gly Ala Arg Ile  
35 40 45

Val Ala Lys Ala Trp Val Asp Pro Gln Phe Arg Glu Leu Leu Leu Lys  
50 55 60

Asp Gly Thr Ala Ala Cys Ala Gln Phe Gly Phe Thr Gly Pro Gln Gly  
65 70 75 80

Glu Tyr Ile Val Ala Leu Glu Asp Thr Pro Gln Leu Lys Asn Val Ile  
85 90 95

Val Cys Ser Leu Cys Ser Cys Thr Asn Trp Pro Val Leu Gly Leu Pro  
100 105 110

Pro Glu Trp Tyr Lys Gly Phe Glu Phe Arg Ala Arg Leu Val Arg Glu  
115 120 125

Gly Arg Thr Val Leu Arg Glu Leu Gly Thr Glu Leu Pro Gly Asp Met  
130 135 140

Val Val Lys Val Trp Asp Thr Ser Ala Glu Ser Arg Tyr Leu Val Leu  
145 150 155 160

Pro Gln Arg Pro Ala Gly Ser Glu His Met Ser Glu Glu Gln Leu Arg  
165 170 175

Gln Leu Val Thr Lys Asp Val Leu Ile Gly Val Ala Leu Pro Arg Val  
180 185 190

Gly

<210> 8  
<211> 220

<212> PRT

<213> Pseudomonas putida

<400> 8

Met Asp Gly Phe His Asp Leu Gly Gly Phe Gln Gly Phe Gly Lys Val  
1 5 10 15

Pro His Arg Ile Asn Ser Leu Ser Tyr Lys Gln Val Phe Lys Gln Asp  
20 25 30

Trp Glu His Leu Ala Tyr Ser Leu Met Phe Ile Gly Val Asp His Leu  
35 40 45

Asn Lys Phe Ser Val Asp Glu Ile Arg His Ala Val Glu Arg Ile Asp  
50 55 60

Val Arg Gln His Val Gly Thr Glu Tyr Tyr Glu Arg Tyr Val Ile Ala  
65 70 75 80

Thr Ala Thr Leu Leu Val Glu Thr Gly Val Ile Thr Gln Ala Glu Leu  
85 90 95

Asp Glu Ala Leu Gly Ser His Phe Lys Leu Ala Asn Pro Ala His Ala  
100 105 110

Gln Gly Arg Ala Ala Ile Ile Gly Arg Ala Pro Phe Glu Val Gly Asp  
115 120 125

Arg Val Ile Val Arg Asp Glu Tyr Val Ala Gly His Val Arg Met Pro  
130 135 140

Ala Tyr Val Arg Gly Lys Gln Gly Val Val Leu His Arg Thr Thr Glu  
145 150 155 160

Gln Trp Pro Phe Pro Asp Ala Ile Gly His Gly Asp Gln Ser Ala Ala  
165 170 175

His Gln Pro Thr Tyr His Val Glu Phe Arg Val Arg Asp Leu Trp Gly  
180 185 190

Asp Ala Ala Asp Asp Gly Leu Val Val Asp Leu Phe Glu Ser Tyr  
195 200 205

Leu Asp Arg Val Glu Ser Pro Arg Val Val Arg Ala  
210 215 220

<210> 9  
 <211> 1089  
 <212> DNA  
 <213> Pseudomonas putida

<220>  
 <221> CDS  
 <222> (1)..(1089)  
 <223> Coding region of activator protein gene

<400> 9		
atg agt gcc ggc gcc cag gca ggc cggtc ctt tca	48	
Met Ser Ala Gly Ala Gln Ala Gly Arg Leu Pro Val Thr Val Leu Ser		
1 5 10 15		
ggc ttc ctc ggc gca ggc aag acc acc ctg ctc aac cac atc ctg cgc	96	
Gly Phe Leu Gly Ala Gly Lys Thr Thr Leu Leu Asn His Ile Leu Arg		
20 25 30		
aac cgc cag ggc ctg aag gtg gcg gtt atc gtc aat gac atg agc gag	144	
Asn Arg Gln Gly Leu Lys Val Ala Val Ile Val Asn Asp Met Ser Glu		
35 40 45		
gtc aac atc gat gcc gcc cag gtc cag cgc gac gtt gcg ctg tat cgt	192	
Val Asn Ile Asp Ala Ala Gln Val Gln Arg Asp Val Ala Leu Tyr Arg		
50 55 60		
ggc cag gat gaa ttg ata gag atg agc aac ggc tgt atc tgc tgc acc	240	
Gly Gln Asp Glu Leu Ile Glu Met Ser Asn Gly Cys Ile Cys Cys Thr		
65 70 75 80		
ctg cgc gcc gac ctg ctt gag cag atc agc gcg ctg gcg cgc cag cag	288	
Leu Arg Ala Asp Leu Leu Glu Gln Ile Ser Ala Leu Ala Arg Gln Gln		
85 90 95		
cgt ttc gat tac ctg ttg atc gag tcc acc ggg att tcc gag ccg atg	336	
Arg Phe Asp Tyr Leu Leu Ile Glu Ser Thr Gly Ile Ser Glu Pro Met		
100 105 110		
cca gtc gcc gag acc ttt gcc ttt ctc gac gcc aac ggt ttc agc ctc	384	
Pro Val Ala Glu Thr Phe Ala Phe Leu Asp Ala Asn Gly Phe Ser Leu		
115 120 125		
agc gaa ctg gcg cgg ctg gat acg ctg gtg acg gtg gtc gat gcc agc	432	
Ser Glu Leu Ala Arg Leu Asp Thr Leu Val Thr Val Val Asp Ala Ser		
130 135 140		
cag ttc atg gcc atg ctc gac tct ccc gaa acc gtc gcg cgg gcc gac	480	
Gln Phe Met Ala Met Leu Asp Ser Pro Glu Thr Val Ala Arg Ala Asp		
145 150 155 160		
gtc acc acg gat gac agc agg cgc ccgtc gac gtc gat ctg ctg atc gag	528	
Val Thr Thr Asp Asp Ser Arg Arg Pro Leu Ala Asp Leu Leu Ile Glu		
165 170 175		
cag gtc gag tat gcc aat gtg att ctg gtc aac aaa cgc gac ctg gtc	576	
Gln Val Glu Tyr Ala Asn Val Ile Leu Val Asn Lys Arg Asp Leu Val		
180 185 190		

gac gag gcg cag tac cag gcc ctg cag gca gtt ctc gcc ggg ctc aat		624	
Asp Glu Ala Gln Tyr Gln Ala Leu Gln Ala Val Leu Ala Gly Leu Asn			
195	200	205	
cca ggc gca cag atc ctg ccg atg gtg gcc ggc aac gtc gcc ctg tcg		672	
Pro Gly Ala Gln Ile Leu Pro Met Val Ala Gly Asn Val Ala Leu Ser			
210	215	220	
agc gtc ctt ggt acc cag ctg ttc gat ttg ccc agc ctt gcc gca gcg		720	
Ser Val Leu Gly Thr Gln Leu Phe Asp Leu Pro Ser Leu Ala Ala Ala			
225	230	235	240
ccc ggc tgg atg aaa cag atg gac gcg cac gac acc ccg gcc ggc gag		768	
Pro Gly Trp Met Lys Gln Met Asp Ala His Asp Thr Pro Ala Gly Glu			
245	250	255	
tcg cag acc tat ggc gtg acg tca tgg gtg tac cga gcg cgc gcc ccg		816	
Ser Gln Thr Tyr Gly Val Thr Ser Trp Val Tyr Arg Ala Arg Ala Pro			
260	265	270	
tcc cat ccg caa cgc ttg ctt gat ttt ctc gcc cgcc ccc tgg cgc gac		864	
Phe His Pro Gln Arg Leu Leu Asp Phe Leu Ala Arg Pro Trp Arg Asp			
275	280	285	
ggc cgt ctt ctg cgc agc aaa ggt tat ttc tgg ctt gcc agc cgc cac		912	
Gly Arg Leu Leu Arg Ser Lys Gly Tyr Phe Trp Leu Ala Ser Arg His			
290	295	300	
cgc gaa atc ggc ttg ctg gta cac agc ggc cag cag ttt caa tgg gac		960	
Arg Glu Ile Gly Leu Leu Val His Ser Gly Gln Gln Phe Gln Trp Asp			
305	310	315	320
tat gtt ggc cat tgg tgg aac ttc atc gac acg tca cag tgg cca cag		1008	
Tyr Val Gly His Trp Trp Asn Phe Ile Asp Thr Ser Gln Trp Pro Gln			
325	330	335	
gac aag tat cgc ttg cag ggc atc atg gcc aag tgg gac agc atc gtc		1056	
Asp Lys Tyr Arg Leu Gln Gly Ile Met Ala Lys Trp Asp Ser Ile Val			
340	345	350	
ggc gac tgc cga cag gag ctg aaa agc tta tga		1089	
Gly Asp Cys Arg Gln Glu Leu Lys Ser Leu			
355	360		

<210> 10  
<211> 362  
<212> PRT  
<213> Pseudomonas putida

<400> 10

Met Ser Ala Gly Ala Gln Ala Gly Arg Leu Pro Val Thr Val Leu Ser  
1 5 10 15

Gly Phe Leu Gly Ala Gly Lys Thr Thr Leu Leu Asn His Ile Leu Arg  
20 25 30

Asn Arg Gln Gly Leu Lys Val Ala Val Ile Val Asn Asp Met Ser Glu

35

40

45

Val Asn Ile Asp Ala Ala Gln Val Gln Arg Asp Val Ala Leu Tyr Arg  
50 55 60

Gly Gln Asp Glu Leu Ile Glu Met Ser Asn Gly Cys Ile Cys Cys Thr  
65 70 75 80  
Leu Arg Ala Asp Leu Leu Glu Gln Ile Ser Ala Leu Ala Arg Gln Gln  
85 90 95

Arg Phe Asp Tyr Leu Leu Ile Glu Ser Thr Gly Ile Ser Glu Pro Met  
100 105 110

Pro Val Ala Glu Thr Phe Ala Phe Leu Asp Ala Asn Gly Phe Ser Leu  
115 120 125

Ser Glu Leu Ala Arg Leu Asp Thr Leu Val Thr Val Val Asp Ala Ser  
130 135 140

Gln Phe Met Ala Met Leu Asp Ser Pro Glu Thr Val Ala Arg Ala Asp  
145 150 155 160

Val Thr Thr Asp Asp Ser Arg Arg Pro Leu Ala Asp Leu Leu Ile Glu  
165 170 175

Gln Val Glu Tyr Ala Asn Val Ile Leu Val Asn Lys Arg Asp Leu Val  
180 185 190

Asp Glu Ala Gln Tyr Gln Ala Leu Gln Ala Val Leu Ala Gly Leu Asn  
195 200 205

Pro Gly Ala Gln Ile Leu Pro Met Val Ala Gly Asn Val Ala Leu Ser  
210 215 220

Ser Val Leu Gly Thr Gln Leu Phe Asp Leu Pro Ser Leu Ala Ala Ala  
225 230 235 240

Pro Gly Trp Met Lys Gln Met Asp Ala His Asp Thr Pro Ala Gly Glu  
245 250 255

Ser Gln Thr Tyr Gly Val Thr Ser Trp Val Tyr Arg Ala Arg Ala Pro  
260 265 270

Phe His Pro Gln Arg Leu Leu Asp Phe Leu Ala Arg Pro Trp Arg Asp  
275 280 285

Gly Arg Leu Leu Arg Ser Lys Gly Tyr Phe Trp Leu Ala Ser Arg His  
290 295 300

Arg Glu Ile Gly Leu Leu Val His Ser Gly Gln Gln Phe Gln Trp Asp  
305 310 315 320

Tyr Val Gly His Trp Trp Asn Phe Ile Asp Thr Ser Gln Trp Pro Gln  
325 330 335  
Asp Lys Tyr Arg Leu Gln Gly Ile Met Ala Lys Trp Asp Ser Ile Val  
340 345 350

Gly Asp Cys Arg Gln Glu Leu Lys Ser Leu  
355 360

<210> 11  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer 1F

<400> 11  
ctccaccata tgagtagc tacttcaacg 30

<210> 12  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer 1R

<400> 12  
tttcataagg ttcttatctcg gatcaaatgg 30

<210> 13  
<211> 25  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer 2F

<400> 13  
atgacggcaa cttcaacccc tggtg 25

<210> 14  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Primer 2R

<400> 14

tcagctcctg tcggcagtgc

20